J Comp Physiol A (1999) 184: 99–106

ORIGINAL PAPER

P. Stušek · K. Hamdorf

Properties of pupil mechanisms in owl-fly *Ascalaphus macaronius* (Neuroptera)

Accepted: 1 October 1998

Abstract Regulation of light flux by pupil mechanisms in the UV-sensitive superposition eye of owl-fly *Ascalaphus macaronius* (Neuroptera) was studied with a fast reflection microspectrophotometric technique. The spectral sensitivity of pupil reaction, which was calculated on the basis of changes of transient amplitude reflection, was almost identical with the one of *Deilephila* eye. This indicates that in spite of different life styles and spectral sensitivities of photoreceptors, pupil closing is triggered by the same photosensitive structure in both eyes. By measuring the spectra of reflected light from the *Ascalaphus* eye between 400 and 700 nm after different dark periods following light stimulation, it was established that the restoration of reflection was much faster in the red than in the blue spectral range. Based on this, we propose that two different pupil mechanisms with different spectral absorption characteristics are involved in light-flux regulation. Fast-reacting pupil is probably represented by screening pigment migration in the secondary pigment cells and a slow blue-absorbing system by the activity in primary pigment cells. The importance of two different pupils for the photoregeneration of visual pigment is discussed.

Key words Pupil spectral activation · Screening pigment migration · Superposition compound eye · Owl-fly *Ascalaphus macaronius* · Insects

Abbreviations $F(\lambda)$ true spectral reflection · $S(\lambda)$ relative spectral sensitivity

P. Stušek (✉)
Department of Biology, Biotechnical Faculty,
Večna pot 111, University of Ljubljana, Slovenia
e-mail: Peter Stušek@uni-lj.si
Fax: 386 61 273390

K. Hamdorf
Institut für Tierphysiologie der Ruhr-Universität,
D-44799 Bochum, Germany

Introduction

In a variety of arthropod eyes, the light flux arriving at the light-sensitive rhabdom of an ommatidium is regulated by migration of screening pigment (Goldsmith and Bernard 1974; Miller 1979). The most remarkable phenomenon accompanied by the pigment migration is the so-called eye glow observed in superposition eyes during dim light. The brightest glow appears when the screening pigment layer is contracted completely between the crystalline cones of the dioptrics. The eye glow is caused by the light reflected from tracheolar baskets surrounding each ommatidial rhabdom. With increasing light intensity, the screening pigment curtain expands gradually and reduces correspondingly the light flux to the rhabdoms with their tracheolar reflectors. The result of this is a gradual diminution of the eye glow. Thus, the amount of reflected light leaving the eye can be used as a measure of the movement of screening pigment granules (Goldsmith and Bernard 1974; Hamdorf et al. 1986). The extent of the pigment expansion is controlled not only by the intensity but also by the wavelength of the stimulating light; furthermore, the molecular processes controlling the pigment migration are strongly dependent on temperature (Nordström and Warrant 1997), on metabolic activity (Weyrauter 1986), and on neuronal hormones (Hamdorf et al. 1989).

The eye glow is observed not only in superposition eyes of animals active in dim light, but also in the superposition eye of the neuropteran *Ascalaphus*, a Mediterranean species which is exclusively active in bright sunshine. The highly specialized superposition eye of *Ascalaphus* is bipartite. Its larger dorsal part, the so called frontal eye, possesses UV receptors only. With respect to UV modulations in the biotope, the frontal eye of *Ascalaphus* is adapted to crepuscular conditions. From the construction of the frontal eye one may assume that the exciting UV flux to the receptors is modulated by pigment expansion in the secondary pigment cells in a similar way as in other superposition eyes.

However, an expansion of pigment granules into the clear zone, such as occurs in typical superposition eyes, could not be observed. Consequently, it was suggested that in the frontal eye the change in eye glow is probably caused by other unidentified pupil or reflecting mechanisms.

In *Ascalaphus*, there are probably three different types of screening pigment involved in modulating the light flux: a yellow pigment and two types of brown pigments. The yellow pigment is located in primary pigment cells and the brown pigment granules are aggregated in distal and proximal parts of the secondary pigment cells as well as in the proximal part of visual cells (Fig. 1; Schneider et al. 1978). The yellow pigment of the primary pigment cells and the brown pigment of the secondary pigment cells are the main candidates for the regulation of the light flux. Of less importance is the dark pigment fixed in the visual cells located close to the basal membrane. Therefore, the question as to whether different types of pigment cells with brown and yellow pigment are involved in regulating the light flux or not, can be answered by measuring the spectra of reflected light during the time-course of light and dark adaptation. The latter was one of the aims of the present study.

The light-sensitive trigger inducing pigment migration can be located in different parts of superposition eyes (Weyrauter 1986; Land 1987; Nilsson et al. 1992). In the sphingid moth *Deilephila*, the trigger is most probably located distally in the eye in the region of the dioptrics. This was proved by two types of experiments: 1) by a local induction of pigment migration with UV-blue light spots within the field of eye glow (Nilsson et al. 1992), and 2) by light induction of pigment migration in the so-called cut preparations, in which the receptors were cut off from the dioptrics at the border of the clear zone. In *Ascalaphus* eye, the trigger for pigment migration must also be located in the distal part of the eye because both types of experiments showed the same results as in *Deilephila* eye (Hamdorf et al. 1989; Nilsson et al. 1992). However, there is some experimental evidence that an additional trigger may exist in the proximal part of the *Ascalaphus* eye (Nilsson et al. 1992).

In *Deilephila* eye, the spectral sensitivity of pigment migration and the spectral sensitivity of photoreceptors differ remarkably (Hamdorf et al. 1986; Nilsson et al. 1992). The sum response of retinula cells in *Deilephila* is dominated by the response of six green receptors (λ_{max} 520 nm), whereas pigment migration is triggered predominantly by UV irradiation (350 nm) and somewhat less by blue light (440 nm) but not by green light.

Surprisingly, in the UV-sensitive superposition eye of *Ascalaphus*, pigment migration is triggered not only by UV but also by blue light. According to this finding it was suggested that in the eyes of *Deilephila* and *Ascalaphus* the trigger for eliciting pigment migration is based on the same photosensitive principle. In order to prove this hypothesis in the present study, the spectral sensitivity of pigment migration in the *Ascalaphus* frontal eye was determined more precisely.

Materials and methods

Animals

Adult owl-flies were caught at the beginning of July in southern parts of Slovenia and kept in the refrigerator at 10 °C. The animals were periodically warmed up and fed with liver. Such care enabled animals to remain in optimal condition for more than 1 month.

Optical measurements

The experimental set-up for the reflection measurements has already been described in detail (Hamdorf and Höglund 1981; Hamdorf et al. 1986, 1989). The animals were immobilized in a conical tube and their heads were fixed to the edge of the tube with Krönigskitt (mixture of wax and collophony, 2:1). The tube was mounted on the microscope object table with plasticine and the eye was adjusted to the light beam. The eye was illuminated via an objective from above and the area of irradiated facets was restricted by a diaphragm positioned in the illuminating beam at the image plane of the objective.

The measuring set-up, based on an epifluorescence microscopic system (Orthoplan, Leitz) was assembled so that the eye could be illuminated by three different light sources via the objective:

1. A tungsten lamp, supplied with d.c. current, used for the observation of the eye in red light and to determine the time-course of reflection changes occurring in the superposition eyes (in most cases measured at 570 nm).
2. An HBO lamp (150 W), used for bright monochromatic adaptations ranging from UV to visible lights. Monochromatic adaptation lights were obtained by interference filters (374 nm, 416 nm, 447 nm, 467 nm, 487 nm, 514 nm and 543 nm). The intensity of the adapting lights measured by thermopile, (Dexter Instruments, using an LD-EPIPLAN 4/1 objective) ranged from 0.31, 5.24, 7.75, 5.88, 6.65 and 2.74 to $24.25 \cdot 10^{18}$ photons $cm^{-2}\ s^{-1}$. For extreme light adaptation, blue light selected by band filter BG 12 was used.
3. An XBO lamp (1600 W) in combination with a computer-controlled monochromator (BM 25, half width of resolution 4 nm), used as chromatic light source for measuring the spectral reflection in the range between 400 nm and 700 nm. The light reflected from the superposition eye was recorded by photon counter (EMI 5862/806). In order to measure only the reflected light from the tapetum layer, the reflection from cornea was excluded by two crossed polarization filters inserted into the optical beam path: one filter was placed in the illumination path and the other one in the measuring path directly in front of the photon counter. The counted photon numbers were stored by an AT computer which also controlled the experimental protocol.

The measuring set-up was calibrated by aluminium oxide placed in the objective plane in place of the eye. The curve of relative spectral light reflection of the white standard is shown in Fig. 5 (curve A).

Electrophysiological measurements

The spectral sensitivity of UV photoreceptors of the frontal eye (Fig. 3, mean value of eight ERGs) was determined by the electrophysiological method described in Hamdorf et al. (1992).

Results

Kinetics and spectral sensitivity of pigment migration

Dark-adapted superposition eyes of *Ascalaphus* show a constant reflection when illuminated with wavelengths

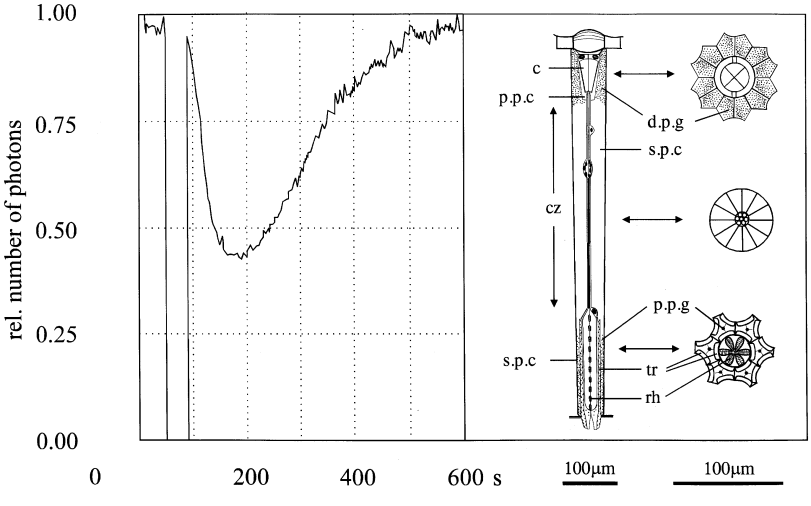
longer than 550 nm. In contrast to this, a brief stimulation with shorter wavelengths cause, after latencies of 20–30 s, a transient change of the reflected light. Figure 1 shows a typical example of such a transient time-course induced by stimulating the eye with light of wavelength 416 nm and of 20 s duration. The amplitude of the reflection minimum (~100 s after stimulation) depends both on stimulus intensity and on stimulus duration and conforms to the $(I \times t)$ law during the period of latency. This relationship between reflection minimum and the $(I \times t)$ of monochromatic stimulation at 447 nm and 416 nm is shown in Fig. 2 where the abscissa was normalized to the number of photons (I_{447}) at 447 nm for 20 s duration (t_{20}): $I_{447} \cdot t_{20} = 1$. Consequently, the relative number of photons at 10 s stimulus duration (t_{10}) is 0.5, at 5 s duration (t_5) is 0.2 and at 2 s duration (t_2) is 0.1. The data for the curve at 416 nm were also normalized to the number of photons at 447 nm for 20 s, which means that the relative number of photons is $\frac{I_{416} \cdot t}{I_{447} \cdot t_{20}}$. The data sets for 447 nm and 416 nm stimuli show the same type of function. Thus, the parallel shift of the 416-nm function is due to a two to three times higher effectiveness of wavelength 416 nm in comparison with 447 nm. Because all other experiments with different monochromatic stimuli (374, 467, 487, 514, 543 nm) were also related to the 447-nm, 20-s stimulus, the distances between the data points and the 447-nm curve correspond to the effectiveness of the wavelengths in respect to 447 nm. Thus, the distances can be used to calculate the relative spectral sensitivity curve of pigment migration ($S(\lambda)$) in the range between 374 and up to 543 nm (see Figs. 3, 9). (Unfortunately, the function $S(\lambda)$ could not be determined in the shorter UV range because the measuring equipment did not allow stimuli shorter than 374 nm to be administered).

The dotted line in Fig. 3 represents the spectral sensitivity of visual cells (left scale) obtained by electrophysiological recordings in this study, and the solid line the spectral sensitivity of screening pigment migration (right scale). The scale of screening pigment migration was normalized to the receptor sensitivity at 374 nm, i.e. to the shortest wavelength used in this study to elicit pigment migration. This normalization was made on the assumption that pigment migration is triggered by UV visual pigment, and so the peaks of both functions have the same λ_{max} values around 345 nm. Thus, this adjustment of the curves is a proof of whether the UV receptors are the only trigger for pigment migration or not. It is obvious that the spectral function of pigment migration deviates significantly from that of the visual cells: pigment migration can be induced not only by UV but also by light of the visual range between 447 nm and up to 514 nm, which does not excite the UV receptors.

Spectral reflection characteristics of the frontal eye

In comparison to the eye glow of sphingid moths active in dim light, the eye glow of *Ascalaphus* is much less conspicuous. Whereas the eye glow of *Deilephila* can be observed at any wavelengths in the visible range, the glow of *Ascalaphus* can better be observed in the blue-green range. The change in reflection of blue-green light can be drastic. Numerous experiments in which this effect was measured gave the same results. A typical example of this kind is shown in Fig. 4 where the spectra

Fig. 1 *Left*: the time-course of reflection change in the frontal eye of *Ascalaphus* (measured at 570 nm) after 20 s monochromatic adaptation (416 nm, 60–80 s). In order to protect the photon counter during adaptation, the shutter in front of the counter was closed. Note the rapid decrease of reflection after stimulation to minimum value at about 180 s and its complete restoration within the subsequent 400 s. *Right*: semi-schematic drawing of a UV-sensitive *Ascalaphus* ommatidium; *c* crystalline cone; *cz* clear zone; *d.p.g* distal pigment granules (brown pigment); *p.p.g* proximal pigment granules (brown pigment); *p.p.c* primary pigment cell (yellow pigment); *s.p.c* secondary pigment cell; *rh* rhabdom; *tr* tracheole. The screening pigment in the primary pigment cells is not indicated. For the sake of clarity, the cross sections of the ommatidium are twice as wide as the width of the longitudinal section. The tracheolar basket is made of six tracheas tightly sealing the proximal third of the hexagonal array of 12 secondary pigment cells which are shared with the adjacent ommatidia. The number of primary pigment cells is two. *Dark bars*: 100 μm for longitudinal and cross sections



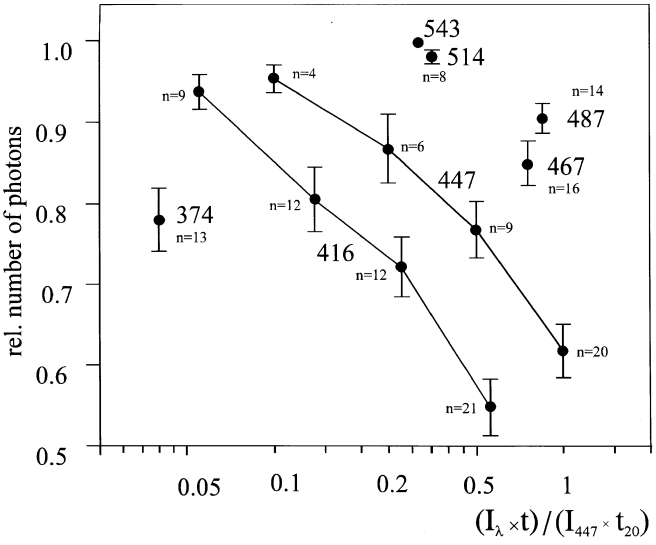


Fig. 2 The amplitude of reflection minimum depending on the applied quanta during stimulation at 447 nm and 416 nm, measured at 570 nm as in Fig. 1. The number of quanta at these two wavelengths was varied by the duration of stimulus between 2 s and 20 s (2, 5, 10 and 20 s). All adaptation experiments at other wavelengths (374, 467, 487, 514 nm), as well as a 20-s stimulus at 416 nm, were normalized to the number of photons at 447 nm (I_{447}) with a 20-s stimulus (t_{20}). This means that $I_{447} \cdot t_{20} = 1$. For all the other wavelengths and stimulus durations the relative number of quanta is $\frac{I_\lambda \cdot t}{I_{447} \cdot t_{20}}$. The number of experimental animals used at different wavelengths and stimulus durations are denoted by *n*; mean values ± standard errors of the mean (*dots with bars*)

of reflected light measured before (the curve labelled as 0) and immediately after stimulation with strong blue light (15 s), as well as during subsequent dark periods (after 4, 9, 13 and 20 min) are represented. The reflection

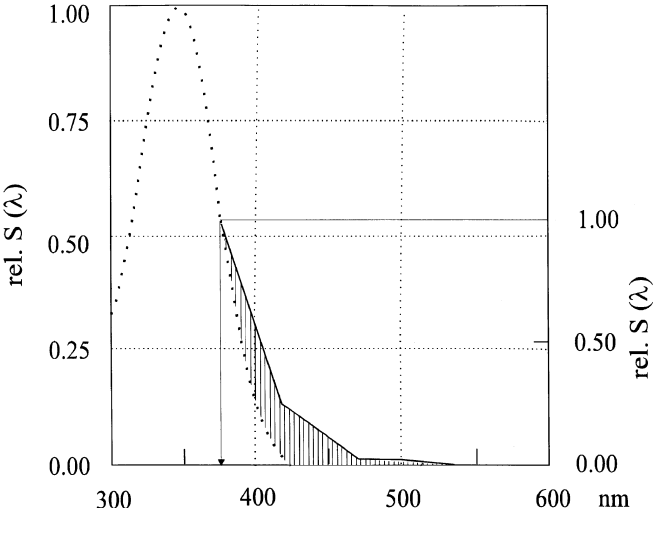


Fig. 3 Relative spectral sensitivity of pigment migration (*right ordinate*, $S(\lambda)$) compared with the relative spectral sensitivity of the UV photoreceptors of *Ascalaphus* frontal eye (*left ordinate*). The ordinate for pigment migration was adjusted to the shortest wavelength, 374 nm, used in the adaptation experiments of Fig. 2. Note the remarkable deviation of the curves in the visual range

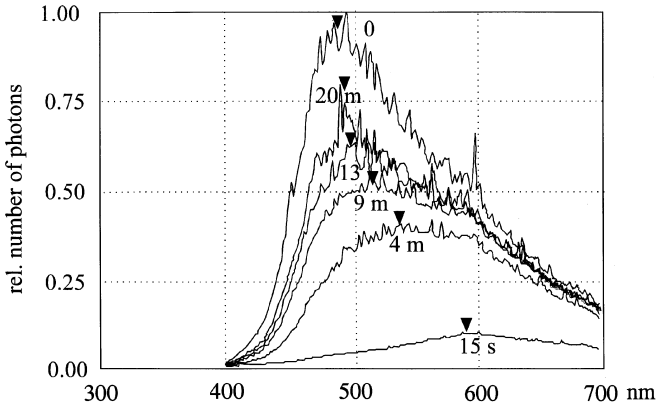


Fig. 4 Actual recording of the spectral reflection before (0) and after (15 s, 4, 9, 13 and 20 min) bright blue adaptation (band filter BG 12; 15 s). Ordinate: number of reflected photons normalized to the spectral maximum of curve 0. Note that a short time after stimulation (curve 15 s) the spectral reflection becomes reduced in the whole measured range between 400 nm and 700 nm and that the peaks of the spectral reflection curves (*arrow heads*) are shifted from 590 to 490 nm during the subsequent periods in the dark

at 490 nm drops immediately after stimulation to less than 5% of the original value in dark. For the complete regeneration of the eye glow in the dark about 1 h is necessary, but to reach the 50% value of regeneration only 9 min are required. The modulation of the reflected red light between 600 and 700 nm is smaller than of the blue-green light between 400 nm and 500 nm. At 650 nm, for example, the intensity of the reflected light is reduced to about 0.25, and furthermore, in the dark the original value is reached relatively fast, i.e. after 9 min.

In parallel with these effects during the dark period, the peaks of the reflection spectra change continuously from ~590 to 490 nm. This shift of λ_{max} in reflection becomes more pronounced by normalizing the curves of light- and dark-adapted states to their maxima (Fig. 5). These two effects extracted from original data in Fig. 4 and Fig. 5, are indications that two different systems are

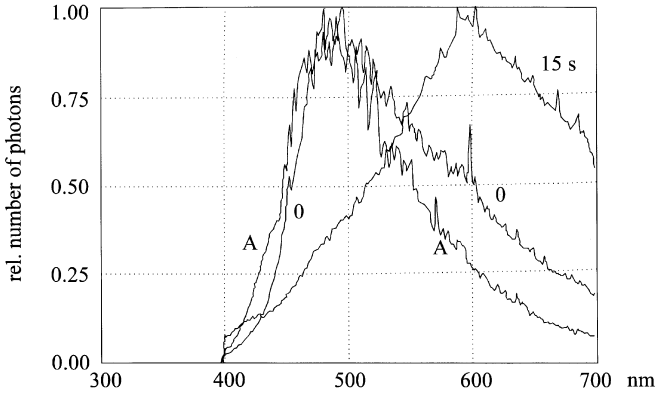


Fig. 5 The shift of the spectral reflection peak caused by light adaptation (blue light BG 12; 15 s) and the reflection of white standard measured under the same experimental conditions. Curves: before stimulation (0), after stimulation (15 s), and white standard (*A*). All curves normalized to their spectral maximum

involved in light flux regulation, one being the predominantly red-absorbing fast system and another, a slower blue-green-absorbing system.

The difference between the speeds of these spectral reactions becomes more pronounced when the original data from Fig. 4 are transformed to the function $\frac{I_{L,t}(\lambda)}{I_D(\lambda)}$ which is independent of the intensity of the spectral light of the monochromator used in our experiments, where $I_D(\lambda)$ is the intensity of the spectral light reflected from the dark-adapted eye and $I_{L,t}(\lambda)$ the intensity of the light reflected from the light-adapted eye at time t after stimulation (Fig. 6). These functions show the relative spectral brightness of eye glow at different times after light stimulation. In the red region the dark value of reflected light is almost reached after 9 min, whereas in the blue region at 450 nm the regeneration process is obviously retarded. Even 20 min after illumination, the reflection at 450 nm remains essentially reduced (Fig. 6, curve 20). Further indication for the existence of a slow-reacting blue-absorbing system is obtained by the transformation of the experimental data as follows: according to Lambert-Beer's law $\left(\text{extinction } E = \log\left(\frac{I_0}{I_E}\right)\right)$, the spectral absorbance of pupil pigment, or other light-absorbing structures functioning as the pupil, is given by $E(\lambda) = \log\left(\frac{I_D(\lambda)}{I_{L,t}(\lambda)}\right)$. Because the blue-absorbing system remains closed longer than the red one, its spectral absorbance can be calculated from difference spectra at times when the red-absorbing system has already recovered. For calculating such difference spectra, ΔE, the following equation can be applied:

$$\Delta E = \log\left(\frac{I_D(\lambda)}{I_{L,t1}(\lambda)}\right) - \log\left(\frac{I_D(\lambda)}{I_{L,t2}(\lambda)}\right) = \log\left(\frac{I_{L,t2}(\lambda)}{I_{L,t1}(\lambda)}\right).$$

Figure 7 shows two such difference spectra where spectrum 1 (between 15 s and 4 min) still includes the absorbance of both light-absorbing systems, whereas spectrum 2 (between 4 min and 20 min) corresponds to the almost pure spectrum of the blue-absorbing system.

True spectral reflection of the frontal eye

In order to ascertain the efficiency of the reflecting eye structures, the spectral reflection of the white standard $\frac{I_A(\lambda)}{I_{A\lambda_{max}}}$ (aluminium oxide, Fig. 5, curve A) was measured under the same conditions as the spectral reflection curves of the dark- (0) and light-adapted (15 s) frontal eye (Fig. 4).

As the relative spectral eye glow is defined by $\frac{I_{L,t}(\lambda)}{I_{D,\lambda_{max}}}$, the true relative spectral reflection of the frontal eye $F(\lambda)$, i.e. its spectral reflection independent of the relative spectral reflection characteristics of the measuring equipment such as the spectral emission of light source, XBO, the spectral sensitivity of the photon counter, and the spectral properties of microscopic optics is given by:

$$F(\lambda) = \frac{I_{L,t}(\lambda)/I_{D\lambda_{max}}}{I_A(\lambda)/I_D(\lambda)/I_{A,\lambda_{max}}}$$

Because the λ_{max} values of I_D and I_A are almost identical (compare curve 0 with curve A, Fig. 5), the spectral F curve of dark-adapted states immediately after bright blue stimulation (15 s) and 4, 9, 13, 20 min later can be calculated from the data of Fig. 6.

The true spectral reflection characteristics of the dark-adapted frontal eye is very similar to that of the white standard (aluminium oxide, Fig. 8). A slightly higher relative reflection can be observed in the red region which exceeds 600 nm, and a slightly lower reflection in the blue range below 480 nm. In the light-adapted state the reflection becomes strongly reduced in the blue range by about 1.5 log units and at longer wavelength ranges by 0.5 log unit. These relatively small changes in $F(\lambda)$ occurring in the dark brown *Ascalaphus* eye during light and dark adaptation are barely

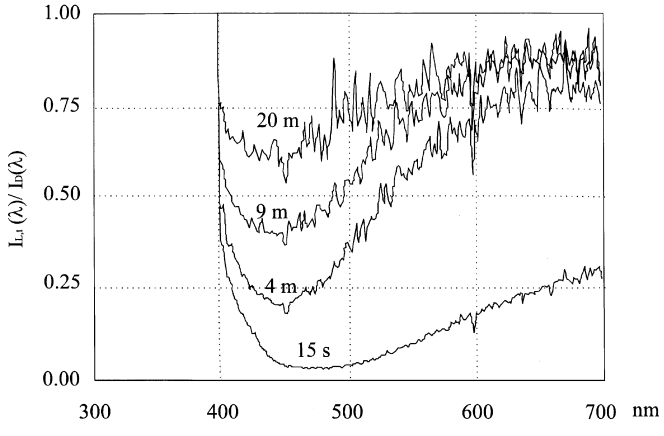


Fig. 6 The relative spectral eye glow of *Ascalaphus* at different times after light adaptation obtained by converting the data of Fig. 4 into the function $\frac{I_{L,t}(\lambda)}{I_D(\lambda)}$. Curves: short after stimulation (15 s) and of subsequent minutes in the dark (4, 9, 20 min)

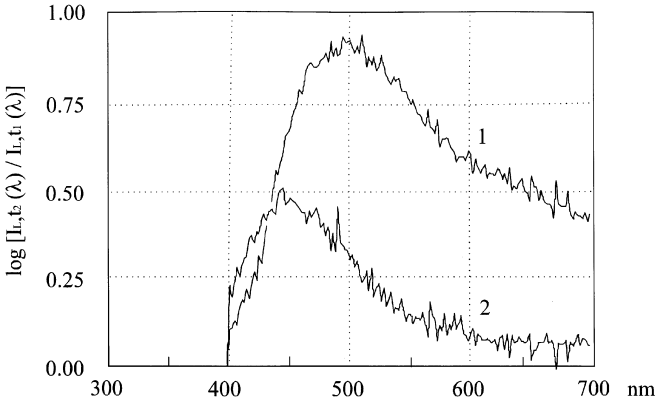


Fig. 7 Difference spectra calculated from data of Fig. 4 between the 15-s curve and 4-min curve (*1*) and between the 4-min curve and 20-min curve (*2*). Curve 2 is due to the spectral absorbance of the slow pupil. Note the differences between the two curves concerning their λ_{max} shift from 500 nm to 450 nm and the much lower absorbance of curve 2 around 600 nm in comparison to curve 1

detectable by the naked eye, and only measurable by sensitive physical instruments.

Discussion

Many arthropod eyes are able to regulate the light flux by migration of screening pigment in primary and secondary pigment cells (Goldsmith and Bernard 1974; Miller 1979; Warrant and McIntyre 1990; Warrant and McIntyre 1996). In superposition compound eyes of arthropods, four different mechanisms of screening pigment migration were found. In insects, two mechanisms in which the pigment granules migrate either in secondary or in primary pigment cells are of special interest. The first belongs to a so-called longitudinal pigment migration mechanism and the second to a cone pigment migration mechanism (Warrant and McIntyre 1996). Both systems, harmonizing the light flux to the photoreceptors of the superposition eyes, are developed in accordance with the light modulation occurring in the biotope during the daily periods of insect activity.

The spectral sensitivity of pupil reaction in *Ascalaphus* differs from the spectral sensitivity of its UV photoreceptors

Because in some moths the spectral sensitivity of photoreceptors matches the spectral sensitivity of pigment migration, it was assumed that in these species the pigment migration is directly controlled by photoreceptors (Weyrauter 1986). However, there is also considerable evidence that in other superposition eyes the screening pigment migration is not induced by the activity of visual cells, but more likely by a special trigger located distally (Hamdorf and Höglund 1981; Hamdorf et al. 1986; Land 1987; Nilsson et al. 1992). Such a distal triggering zone was also found in the frontal eye of the neuropteran *Ascalaphus*. Light-sensitive structures responsible for this reaction are still unknown. They can be located either in the thin processes of the photoreceptors contacting the crystalline cone, or in the primary pigment cells themselves, or at least in the distal parts of the secondary pigment cells (Fig. 1).

In order to determine the spectral sensitivity of pigment migration in *Ascalaphus*, we chose the method described in detail by Hamdorf et al. (1986), which differs from the method used by Nilsson et al. (1992). The simpler technique applied here is adequate to answer the question of whether the trigger chromophore for pigment migration coincides with the UV visual pigment or not (Figs. 2, 3, 9). The comparison of the spectral sensitivities of UV photoreceptors and of the light-induced pupil response show a clear difference in the range exceeding 400 nm (Fig. 3). Thus, one cannot assume that the light reflection changes in the frontal eye are triggered by UV photoreceptors alone, therefore, the idea of a special triggering system located near the dioptrics of the eye becomes more reliable.

In spite of different light environments, pupil responses in *Ascalaphus* and *Deilephila* are probably triggered by an identical sensor chromophore

The data points of the *Ascalaphus* pupil sensitivity function match surprisingly well the spectral sensitivity function of the sphingid moth *Deilephila elpenor* (see Fig. 9). This finding indicates that in the eyes of both species, the screening pigment migration is probably controlled by a similar or identical sensor chromophore. In both species, this chromophore is located in the distal

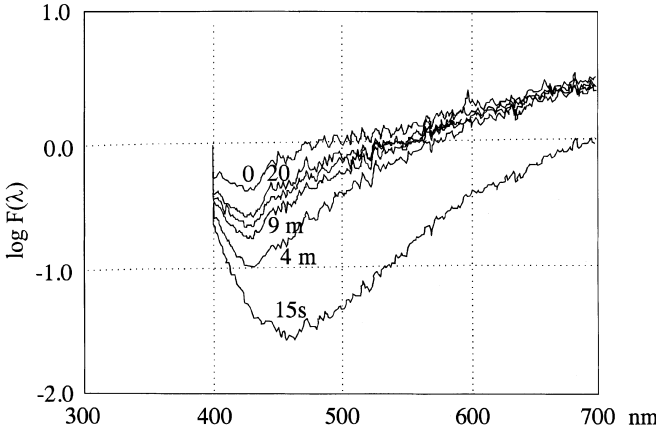


Fig. 8 The spectral reflectance of dark-adapted and of light-adapted eyes at different times after stimulation normalized to the white reflection standard (aluminium oxide). Curves of 15 s, 4, 9, 13, and 20 min are based on data from Fig. 4. For an explanation of ordinate log $F(\lambda)$ see text

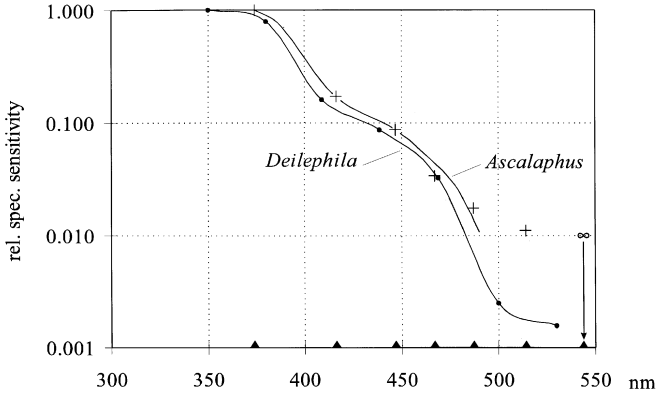


Fig. 9 Comparison of the relative spectral sensitivities of pigment migration in superposition eye of *Deilephila* (*dots*) and in the frontal eye of *Ascalaphus* (*crosses*). Data of *Deilephila* after Nilsson et al. 1992. Note that in the approximation of the function, the calculated values for 514-nm and 543-nm stimulation of Fig. 2 were excluded, because the technique applied here does not allow a more precise calculation of $S(\lambda) \leq 0.01$

part of the eye close to the dioptrics, as has already been shown by light spot experiments (Nilsson et al. 1992) and by cut preparations (Hamdorf et al. 1986, 1989). Furthermore, the parameters of the time-courses of pigment migration are also very similar in both superposition eyes (see Fig. 1 this study and Fig. 1 in Hamdorf et al. 1986) indicating that the molecular reaction mechanisms of pigment expansion and contraction are likely the same.

At first glance, this similarity of physiological data is surprising because the two species are adapted to entirely different environments. *Ascalaphus* is a typical diurnal animal of Mediterranean meadows exposed to bright sunlight, whereas *Deilephila* is a crepuscular animal active only in dim light. However, with respect to their different photoreceptor system, the light conditions in their biotopes are not as different as they seem. The amount of UV emitted by the sky and available for UV photoreceptors is in general much lower than the amount of radiation in the visible range exceeding 400 nm, and in addition, during the day time and by clouds, the amount of UV is more strongly modulated than the amount of visible light. The *Ascalaphus* eye has to be adapted to these lower UV intensities, as well as to their large modulations, in a similar way to other superposition eyes of crepuscular species active at dawn or dusk, when the intensities are changing drastically.

In consequence of these environmental conditions and of the above-mentioned morphological similarities, one would expect that the *Ascalaphus* eye would employ a longitudinal pigment migration mechanism, as established for the *Deilephila* eye (Höglund 1966). Earlier LM and EM studies on longitudinal sections through the dorsal part of the eye seem to support these supposition (Schneider et al. 1978). However, recent observations do not confirm these findings (Drašlar 1998), i.e. the position of the pigment granules of the secondary pigment cells inspected by SEM remains constant, irrespective of light- or dark-adapted states (K. Drašlar, unpublished observations). These observations indicate that in spite of apparently the same pigment migration trigger the pupil mechanisms of *Ascalaphus* and *Deilephila* eyes may differ essentially. This conclusion is further supported by the spectral reflection measurements of this study.

In *Ascalaphus* eye the light flux is regulated by fast- and slow-reacting pupil

After bright blue stimulation for a few seconds, the spectral reflection becomes reduced in the whole spectral range (see Figs. 4, 6). During subsequent minutes in the dark, the reflection is gradually restored; however, in the red range between 600 nm and 700 nm the reflection reappears much faster than in the blue range around 450 nm. In parallel to this effect, the λ_{max} values of relative reflected photons shift from longer to shorter wavelengths by about 100 nm (Figs. 4, 5). Thus, the dark-adaptation process exhibits two steps: a faster and a slower one. This phenomenon can be explained quite simply if two distal pupil mechanisms are involved.[1]

In the case of two distal pupil mechanisms, the spectral absorbance of the slow pupil can be evaluated from the difference spectra of Fig. 7. The difference spectrum, curve 1, showing the spectral change during the first few minutes after light stimulation, is mainly due to the spectral characteristics of the fast-reacting pupil. The difference spectrum between the 4^{th} and the 20^{th} min in the dark, curve 2, represents the slow spectral change after the first fast period of dark adaptation, i.e. after opening of the fast pupil. Thus, curve 2 corresponds to the spectral absorbance of the slow pupil which absorbs selectively in the shorter wavelength region. This pupil must possess a blue-absorbing pigment. The only cell type in the eye which accumulates yellow pigment of similar blue absorption characteristics, is the primary pigment cell (Schneider et al. 1978). Therefore, the blue-light modulation caused by the slow pupil is most probably due to the yellow pigment in primary pigment cells acting as a cone-pupil mechanism, similar to that described for the *Copris* superposition eye (Scarabeidae; Warrant and McIntyre 1990, 1996).

The spectral characteristics of the fast pupil mechanism are much less specific than that of the slower one. The fast pupil reduces the reflected light over the whole range of the measured spectrum (from 400 to 700 nm), as indicated by the comparison of curves 15 s and 4 min in Fig. 6 and of curves 1 and 2 in Fig. 7. For this reason, the fast pupil acts in a similar manner to a neutral grey filter wedge. Such neutral reduction of all reflected wavelengths is typical of superposition eyes with a longitudinal pigment migration mechanism where the brown-black pigment granules of the secondary pigment cells cut off all wavelengths equally. Consequently, the intensity of reflected light originating from the rhabdoms is reduced by the expansion of the black pigment curtain in the eye.

Less conspicuous eye glow in *Ascalaphus* is probable due to a very small pigment displacement in secondary pigment cells

The colour of *Ascalaphus* eye is always dark brownish, no matter whether it is light or dark adapted. This indicates that the colour of the eye is dominated by the absorption characteristics of the secondary pigment cells, and also that even in the dark-adapted state, the secondary pigment of *Ascalaphus* eye is less contracted than in other superposition eyes that show bright eye glow. The comparison between the spectral reflections of the *Ascalaphus*

[1]However, if only one distal pupil is regulating the light flux, then one has to postulate that its dark-adaptation process either occurs in two consecutive steps, or that, in addition to distal pupils or pupil, other unidentified fast-reacting structures modulating the reflected light are existent, e.g. a fast-reacting screening pigment in the proximal part of the eye.

eye and a white reflection standard is in line with human colour sensation (see Fig. 8). Because the reflected light between 550 nm and 700 nm remains almost constant, irrespective of the small reflection changes in the blue-green (compare curves 0 to 4), a colour change will not be perceptible. It is only immediately after light adaptation that the eye glow seems to be darker to the observer, but the colour is not seen to change because, as before, light in the long-wavelength range is almost proportionally reduced (compare 15-s curve with other curves). This short-term effect must be due to an almost neutral spectral filter, probably produced by an expansion of screening pigment granules in the secondary pigment cells where the pigment displacement is considered as longitudinal pigment mechanism.

Since the efficiency of longitudinal pupils is in general much higher than of the cone pupils, an expansion of only 10% of the possible maximum expansion reduces the eye glow down to 25%, and 30% expansion reduces the eye glow to zero (Warrant and McIntyre 1996). Therefore, the change of up to 25% of the eye glow in *Ascalaphus* measured at 650 nm (see Fig. 6, curve 15 s), would correspond to a small pigment expansion of only 10% in the *Agrotis* eye, which has similar dimensions of dioptric layer and clear zone. Thus, it is to be expected that in the *Ascalaphus* eye a small expansion of the longitudinal pigment curtain by about 10–20 μm would lead to the observed reduction of reflected light. In order to detect and to corroborate such small changes, a very careful histological study is required. Since it is very difficult to carry out such a precise histological evaluation, this can be one of the reasons why, in microscopic studies, no massive displacement of screening pigment into the clear zone was observed.

On the contrary, when *Deilephila* eye becomes light adapted, a drastic reflection change occurs almost equally in the total visual range from 400 nm up to 700 nm. These qualitative differences imply that in *Ascalaphus*, another type of granule acts as a blue-green selective filter while in *Deilephila*, the brown granules of the secondary pigment cells act as a neutral grey filter cutting off all wavelengths almost equally. Because blue-green absorbing granules are found only in the primary pigment cells, these cells must be largely responsible for the pupil effects in *Ascalaphus* eye.

Dual pupil response could promote the photoreconversion of visual pigment

A dual pigment migration mechanism with yellow granules in the primary pigment cells and dark brown granules in the secondary pigment cells indicate a very high degree of specialization of the *Ascalaphus* eye in adapting not only to light conditions in the biotope but also to its photoreconvertible UV rhodopsin-metarhodopsin system. UV light flux to the UV receptors can be precisely regulated by the yellow cone pupil. Unfortunately, using our measuring technique, the spectral absorbance of slow pupil could not be determined in the UV range. However, microspectrophotometric measurements have shown that the yellow pigment granules have a high UV absorption (Schneider et al. 1978). Thus, the light in the range between 500 and 700 nm is hardly absorbed by the yellow pupil. Because this light will be almost unaffected by the cone pigment mechanism, this suggests that the light in this spectral range will contribute to a superposition image at the UV rhabdoms of *Ascalaphus*, as in other superposition eyes. Consequently, the rhabdoms will always be illuminated much more brightly by the light in this range than by UV. Thus, the photoreconversion of the blue-green absorbing metarhodopsin is always promoted by this special design of *Ascalaphus* dioptrics. Particularly in bright sunshine, when the UV flux to the photoreceptors is reduced by the yellow pupil, the intensity of the superimposed reconverting light strongly exceeds the intensity of the exciting UV.

References

Drašlar K (1999) Screening pigment distribution in outer segment of *Ascalaphus macaronius* eye and its function in dark-light adaptation. Computer Assisted Microscopy (in press)

Goldsmith TH, Bernard GD (1974) The visual system of insecta, vol. 2. In: Rochstein M (ed) The physiology of insects. Academic Press, San Francisco, pp 165–172

Hamdorf K, Höglund G (1981) Light-induced retinal screening pigment migration independent of visual cell activity. J Comp Physiol 143: 305–309

Hamdorf K, Höglund G, Juse A (1986) Ultraviolet- and blue-induced migration of screening pigment in the retina of the moth *Deilephila elpenor*. J Comp Physiol A 159: 353–362

Hamdorf K, Höglund G, Juse A, Stušek P (1989) Effect of neurotransmiters on movement of screening pigment in insect superposition eyes. Z Naturforsch 44c: 992–998

Hamdorf K, Hohstrate P, Höglund G, Moser M, Sperber S, Schlecht P (1992) Ultraviolet-sensitizing pigment in blowfly photoreceptor R1-6; probable nature and binding sites. J Comp Physiol A 171: 601–615

Höglund G (1966) Pigment migration, light screening and receptor sensitivity in the compound eye of nocturnal Lepidoptera. Acta Physiol Scand [Suppl] 282: 1–56

Land MF (1987) Screening pigment migration in a sphingid moth is triggered by light near the cornea. J Comp Physiol A 160: 355–357

Miller WH (1979) Intraocular filters. In: Autrum H (ed) Vision in invertebrates. Handbook of sensory physiology, vol VII/6A. Springer, Berlin Heidelberg New York, pp 69–134

Nilsson D-E, Hamdorf K, Höglund G (1992) Localization of the pupil trigger in insect superposition eyes. J Comp Physiol A 170: 217–226

Nordström P, Warrant EJ (1997) The influence of temperature on the pupil mechanism in the eye of turnip moth *Agrotis segetum*. In: Elsner N, Wässle T (eds) Proceedings of 25th Göttingen Neurobiology Conference, vol II. Thieme Stuttgart New York, p 452

Schneider L, Gogala M, Drašlar K, Langer H, Schlecht P (1978) Feinstruktur und Schirmpigment-Eigenschaften der Ommatidien des Doppelauges von *Ascalaphus* (Insecta, Neuroptera). Cytobiologie 16: 274–307

Warrant EJ, McIntyre PD (1990) Limitations to resolution in superposition eyes. J Comp Physiol A 167: 785–803

Warrant EJ, McIntyre PD (1996) The visual ecology of pupil action in superposition eyes. J Comp Physiol A 178: 75–90

Weyrauter E (1986) Do retinula cells trigger the screening pigment migration in the eye of moth *Ephestia kuehniella*? J Comp Physiol A 159: 55–60